Lymphoid Organ Size Varies Among Inbred Lines 6_3 and 7_2 and Their Thirteen Recombinant Congenic Strains of Chickens with the Same Major Histocompatibility Complex

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ABSTRACT The objective was to evaluate lymphoid organ size in chickens from a series of 13 recombinant congenic strains (RCS) and their highly inbred parental lines (6_3 and 7_2). The parental line 6_3 was selected for resistance to tumors induced by Marek's disease virus and avian leukosis viruses, whereas line 7_2 was selected for susceptibility to these tumors. Each RCS on the average contains a random one-eighth of genome from the donor line 7_2. Previous studies have shown that lines 6_3 and 7_2 differ in the size of primary lymphoid organs; i.e., the bursa of Fabricius (BF) and the lobes of the thymus (T) are smaller in line 6_3 than line 7_2. In the current study, the relative size of the T, BF, and spleen was first examined in about 15 males from each of 13 RCS and the 2 parental lines at 60 to 69 d of age. The differences of relative BF, T, and spleen size among the RCS and the parental lines 6_3 and 7_2 differed significantly ($P < 0.001$). Males and females from 4 RCS and the 2 parental lines were evaluated a second time, and differences in the relative sizes in lymphoid organs among the RCS and parental lines were consistent. In 2 RCS, the size of the T and BF was comparatively large as in line 7_2, leading to the conclusion that different allelic forms at 1 or more loci in these RCS regulate the size of both organs. In 2 other RCS, the BF was large compared with the T, suggesting that allelic forms at some loci in these RCS influence the BF independent of the T. The relative lymphoid organ size among the RCS appeared to cosegregate with the concentration of IgG in the plasma measured previously. The evaluation of genomic variability of these lines is underway, and the RCS are available for research on traits that differ between lines 6_3 and 7_2.

Key words: bursa of Fabricius, disease resistance, immunoglobulin G, recombinant congenic strain, thymus

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INTRODUCTION

The chicken was instrumental in demonstrating the development of the thymus (**T**) and bursa of Fabricius (**BF**) as central lymphoid organs responsible for the development and maturation of lymphocytes. The BF-dependent B-cell system is responsible for humoral antibody responses, whereas the T-dependent T-cell system is responsible for cell-mediated immune functions (Good et al., 1966; Good, 1971; Glick, 1979). During evaluation of the T and BF, the size of these organs was noted to differ between different breeds (Glick et al., 1956; Jaap, 1958). Because there was evidence that breeds differed in disease resistance (Hutt, 1958), Glick (1956) and Jaap (1958) hypothesized that an increased size in the BF of White Leghorns was correlated with disease resistance. Subsequently, several investigators selected strains with large or small BF to evaluate heritability of BF weight and functional differences in immune responses, e.g., Glick and Dreesen (1967), using New Hampshires, and Muir and Jaap (1969), using a randombred Barred Rock and an F2 cross of the Barred Rock with a randombred White Rock. In the latter study, the heritability of BF size was impressively high in the Barred Rock (0.61) and F_2 chickens (0.78). This suggests that a limited number of genes influence the size of the BF.

The size of the BF has also been evaluated in strains within breeds selected for immune response or disease resistance. In White Leghorn strains selected for antibody response to SRBC, a high antibody response line had a larger BF but a smaller T than the low line (Ubosi et al., 1985). The size of the BF and T has also been evaluated in White Leghorn lines that were highly inbred during selection for resistance (line 6_3) or susceptibility (line 7_2) to lymphoid tumors [i.e., Marek's disease (**MD**) caused by a MD herpesvirus] and lymphoid leukosis and other neoplasms induced by an avian leukosis retrovirus (Bacon et al., 2000). Interestingly, the line 6_3 chickens have a smaller BF, T, and spleen than 7_2 chickens (Lee et al., 1981; Powell et al., 1982). Lines 6_3 and 7_2 have the same common *B**2 *MHC* haplotype (Hunt and Fulton, 1998;

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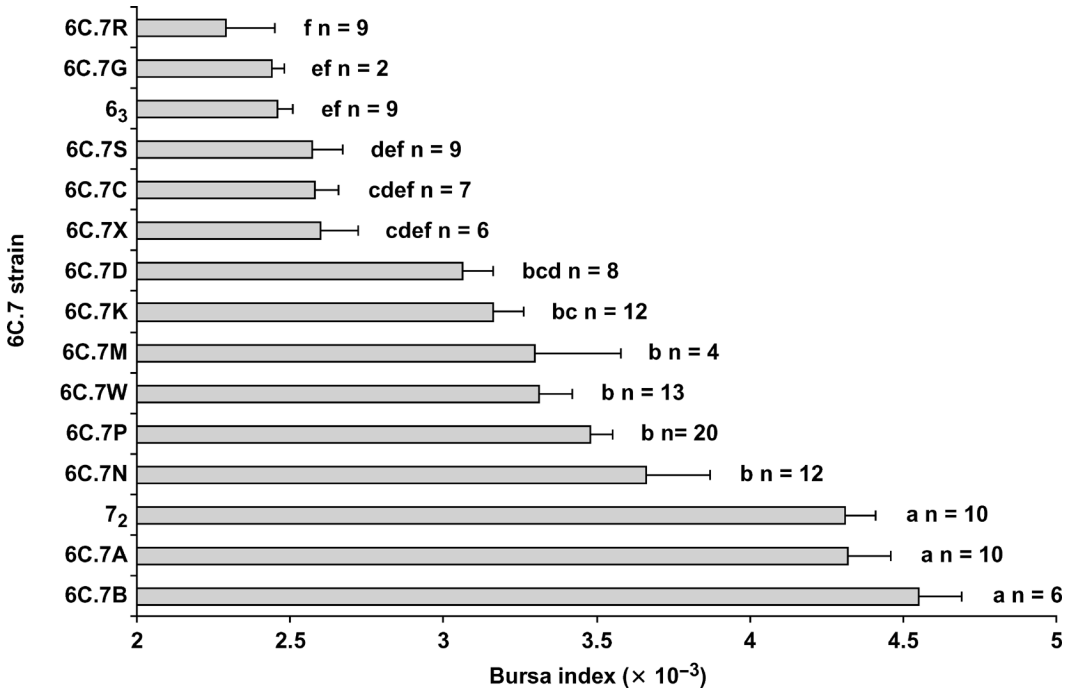

Figure 1. Relative bursa sizes of inbred lines 6_3, 7_2, and 13 recombinant congenic strains. The bursa index was defined as bursa/body weight ratio multiplied by 1,000. Each bar represents the average bursa index with a standard error bar of a line or a strain. The n defines the number of chickens included in the computation of the average index for a line or a strain. [a–f]Bars with no common letter are significantly different ($P < 0.05$).

Pharr et al., 1998), and therefore traits that differ between these lines are attributable to non*MHC* genes.

Nineteen recombinant congenic strains (**RCS**) of chickens are under development originally started by crossing inbred lines 6_3 and 7_2 at the Avian Disease and Oncology Laboratory, (East Lansing, MI). The F_1 males and males from the first backcross were both backcrossed to the parental line 6_3 hens, respectively. Then the RCS were

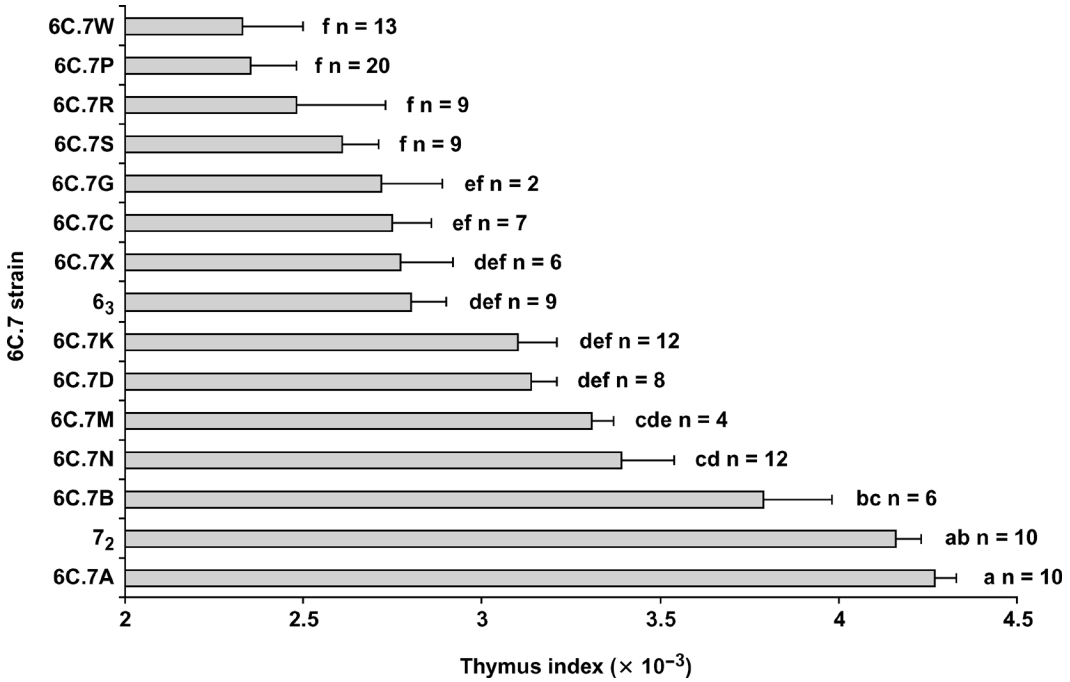

Figure 2. Relative thymus sizes of inbred lines 6_3, 7_2, and 13 recombinant congenic strains. The thymus index was defined as thymus/body weight ratio multiplied by 1,000. Each bar represents the average thymus index with a standard error bar of a line or a strain. The n defines the number of chickens included in the computation of the average index for a line or a strain. [a–f]Bars with no common letter are significantly different ($P < 0.05$).

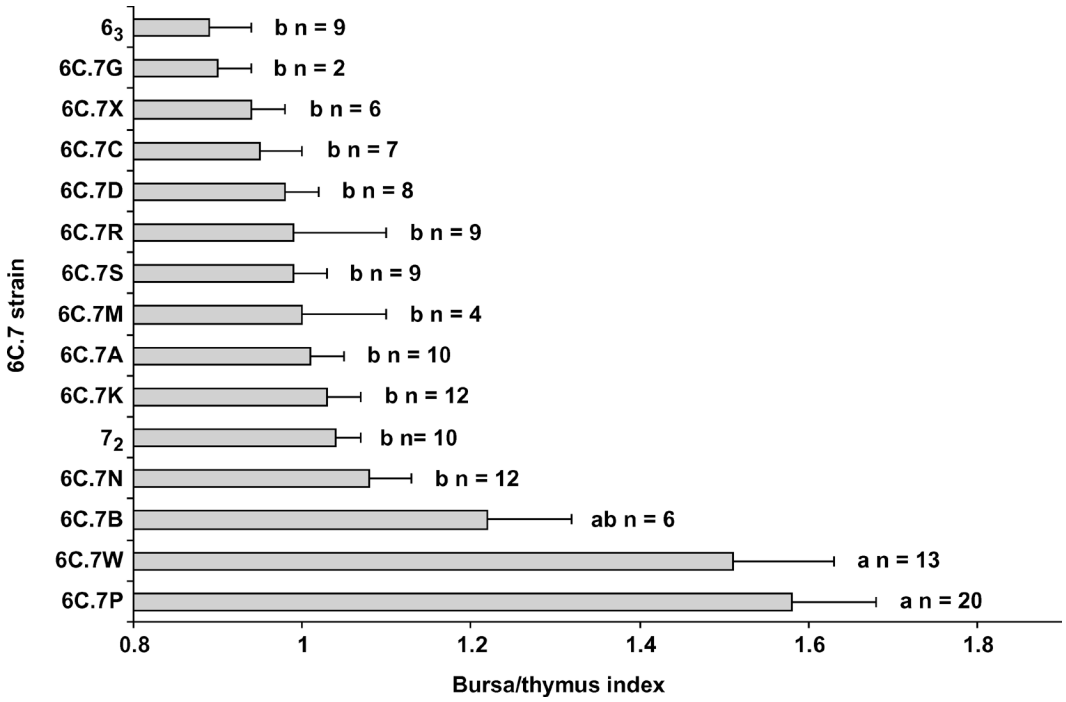

Figure 3. Relative bursa/thymus index of inbred lines 6_3, 7_2, and 13 recombinant congenic strains. Each bar represents the average bursa/thymus index with a standard error bar of a line or a strain. The n defines the number of chickens included in the computation of the average index for a line or a strain. [a,b]Bars with no common letter are significantly different ($P < 0.05$).

formed by sib-matings of the second backcross chickens (Bacon et al., 2000). Each of the RCS consists of a random ⅛ sample of the line 7_2 genome and 7/8 sample of genome from the recurrent female parental line 6_3 (Bacon et al., 2000). The development of the RCS allows for the transformation of a polygenic compound trait into a series of single gene traits (Demant et al., 1989; Yonash et al., 2002). These RCS are now available for evaluation of non*MHC* genes influencing traits differing between lines 6_3 and 7_2, e.g., immunoglobulin G levels (Yonash et al., 2002),

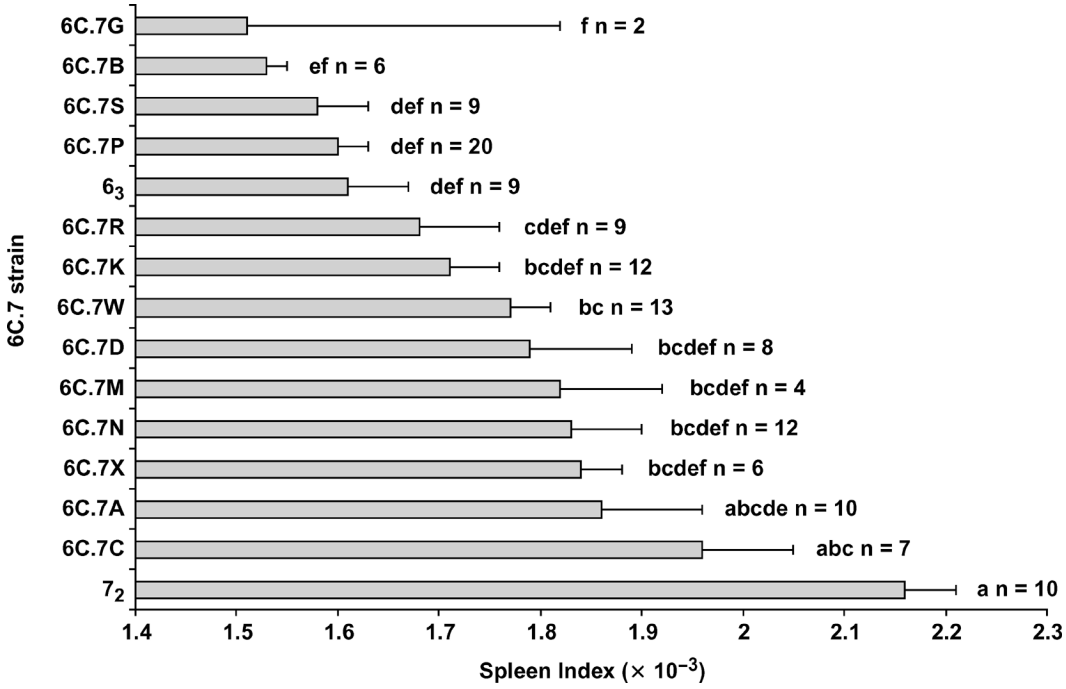

Figure 4. Relative spleen sizes of inbred lines 6_3, 7_2, and 13 recombinant congenic strains. The spleen index was defined as spleen/body weight ratio multiplied by 1,000. Each bar represents the average spleen index with a standard error bar of a line or a strain. The n defines the number of chickens included in the computation of the average index for a line or a strain. [a–f]Bars with no common letter are significantly different ($P < 0.05$).

interferon activity (Bacon and Palmquist, 2002), tumor resistance, and BF or T size.

The purpose of the current study was to simultaneously examine phenotypic variations in the relative weights of BF, T, and spleen in parental lines 6_3 and 7_2, and in 15 RCS. In an exploratory experiment using males 60 to 69 d of age 2 of the RCS were shown to have BF and T organs comparable to 7_2, whereas these organs were smaller and comparable to line 6_3 in the majority of the RCS. These results were replicated in a confirmatory experiment using chickens from both parental lines and 4 RCS at 20, 40, and 60 d of age. Identified RCS should be useful for defining genes that determine BF and T size, and for evaluating correlations between genes determining BF or T size and traits for disease resistance or immune function that differ between inbred lines 6_3 and 7_2.

MATERIALS AND METHODS

Chicken Lines

The ADOL lines 6_3 and 7_2 and 15 RCS (Bacon et al., 2000) were used in the current study. The lines 6_3 and 7_2 are highly inbred sharing a common *MHC* (*B2*) type yet distinctively different in disease resistance including to MD. The RCS were developed using line 6_3 as the recurrent parent for initial crossing and 2 sequential backcross matings. A 6C.7 is used to designate each RCS. The 6 represents line 6_3 as the recurrent parent line; the C indicates a congenic line; and the 7 represents the donor line 7_2. A final capital letter indicates the full-sib family a strain represents.

Organ Extraction

The gross weight of each bird was recorded. The birds were then gassed with carbon dioxide for approximately 5 to 10 min. The chickens were then soaked in a detergent solution to minimize diffusion of chicken dander. Each excised BF was placed in a tube containing 5 mL of Lebowicz McCoy medium with serum so cells could be extracted for another study. The spleen and T lobes were placed in tubes containing 5 mL of phosphate buffered saline solution. Each chicken generally possessed 14 lobes of T, 7 along each of 2 jugular veins. Each tube was weighed and recorded to the nearest 0.1 g prior to and after addition of lymphoid tissue, and the tare weight was recorded for analysis.

Statistical Analyses

To standardize the organ and body weights of the birds due to age differences in 2002, only data from birds between 60 and 69 d of age were included in the analyses. A GLM procedure was used to perform ANOVA to ensure that there were no age-related differences between the strains at 60 to 69 d of age ($P = 0.0633$).

Four indices were defined to evaluate the relative sizes of the lymphoid organs. The BF index, T index, BF/T index, and spleen index were calculated as ratio of BF/body weight, T/body weight, BF/T weight, and spleen/body weight, respectively. A Levene's homogeneity of variance test was conducted to determine if a transformation of the data was needed before an ANOVA could be performed (SAS Inst., 1995). The dependent variables spleen and BF/T index needed no transformation. The variance of the dependent variable BF index was stabilized using the transformation of arcsine (BF weight/body weight)$^{1/2}$, and the T index variance was stabilized by the (T weight/body weight)^2 transformation.

An analysis of covariance was performed with treatments consisting of the inbred line and different RCS chickens and the covariate being age in days for the dependent variables mentioned previously for data collected from the year 2002 chickens (SAS Inst., 1995). The ANOVA showed there was no significant difference between relative lymphoid organ size in males and females in the 2004 chickens except for the BF ($P < 0.05$), and therefore data of males and females were pooled for subsequent analyses. When a significant *F*-test result for treatment was obtained from the ANOVA, Duncan's multiple range tests were performed to determine pairwise treatment differences. Data in figures and tables are presented as raw data for ease of interpretation even though all analyses were performed on transformed data where appropriate. Pearson's correlation coefficients were computed to evaluate the covariation between the average indices of the primary lymphoid organs and the average levels of IgG expression previously measured in 3 separate generations of lines 6_3, 7_2, and the RCS (Yonash et al., 2002).

Experimental Design

Experiments were conducted in 2002 and 2004. Both utilized completely randomized designs with unequal numbers of birds for each treatment (line and RCS).

2002 Experiment. Limited numbers of hens were available in each 6C.7 RCS, and thus it was not possible to obtain about 15 chickens of each line for analysis of a trait. Therefore, excess breeder males were used from each of several hatches for evaluating lymphoid organ size. These chickens were all housed in the same room in cages containing chicks of several lines. At hatch the chicks were vaccinated with turkey herpesvirus MD vaccine (Witter et al., 1970). Males were obtained at about 7 to 10 wk of age from 15 of the 6C.7 RCS and lines 6_3 and 7_2. The age at termination was recorded along with weight data. Data from all hatches were pooled for statistical analysis.

2004 Experiment. Based on the 2002 results chickens from lines 6_3 and 7_2, and 4 6C.7 RCS, i.e., 6C.7B, 6C.7P, 6C.7W, and 6C.7X were selected for repeat analysis. Ten chickens of each line were hatched in 3 hatches. The chickens were vaccinated with turkey herpesvirus at hatch, and each cage contained chickens of all lines. All were caged in the same room. Chickens from both sexes were analyzed for organ weight. One hatch group was termi-

Table 1. Correlation coefficients between average indices of the primary lymphoid organs and the average levels of IgG expression across 2 inbred lines and their 13 recombinant congenic strains (RCS)[1]

	Average level of IgG expression		
Lymphoid organ	Generation H	Generation I	Generation J
Average bursa index	0.45	0.76**	0.57*
Average thymus index	0.60*	0.79**	0.69**

[1]The average primary lymphoid organ index was calculated from the 2002 data of lines 6_3, 7_2, and the 13 RCS. The average levels of IgG expression were from data measured in 3 generations (Yonash et al., 2002).

*Indicates the correlation coefficient estimate was greater than zero, $P < 0.05$. **Indicates the correlation coefficient was greater than zero, $P < 0.01$.

nated at 20, 40, and 60 d of age to observe ontogeny and repeatability of results.

RESULTS

Analysis of Lymphoid Organ Weights in 2002 for Lines 6_3, 7_2, and 13 RCS

The relative weight ratio for the spleen, BF, and T, presented as an index, and the BF/T index, had significant variation ($P < 0.001$) among the ADOL lines 6_3, 7_2, and thirteen 6C.7 RCS. The BF weight was evaluated in males 60 to 69 d of age (Figure 1). Line 7_2 had a significantly larger relative BF than line 6_3. Only 2 RCS had a relatively large BF comparable to line 7_2 (6C.7A and 6C.7B). Five RCS had a relative BF weight that did not differ from line 6_3 (6C.7R, -G, -S, -C, -X). An additional 6 RCS had a relative BF size intermediate between lines 6_3 and 7_2. Line 7_2 had a significantly larger relative T weight at 60 to 69 d of age than line 6_3 (Figure 2). Again the same 2 RCS were comparable to line 7_2 (i.e., 6C.7A and 6C.7B). The remaining 11 RCS did not differ significantly from line 6_3 in relative T weight. Lines 6_3 and 7_2 and 11 RCS had BF/T indices that did not differ significantly (Figure 3). Interestingly, 2 RCS (6C.7P and 6C.7W) had a greater BF/T index that was significantly greater than 10 of the RCS as well as lines 6_3 and 7_2. Line 7_2 had a significantly larger relative spleen size than line 6_3 (Figure 4). The 6C.7C was the only RCS with a relative spleen size comparable to line 7_2 and larger than line 6_3. The remaining 12 RCS had a relative spleen size comparable to line 6_3.

Correlation Between Primary Lymphoid Organ Weight and IgG Level

Significant correlations between the average BF indices and average IgG levels across the inbred lines 6_3, 7_2, and the 13 RCS existed and correlation coefficients ranged from 0.45 to 0.76 (Table 1). Similar correlations were observed between the average T indices and average IgG levels among the same groups of chickens and coefficients ranged from 0.60 to 0.79.

Analysis of Lymphoid Organ Weights in 2004 for Lines 6_3, 7_2, and 4 RCS

The relative BF weight was evaluated at 20, 40, and 60 d of age for 4 selected RCS and the 2 parental lines (Figure

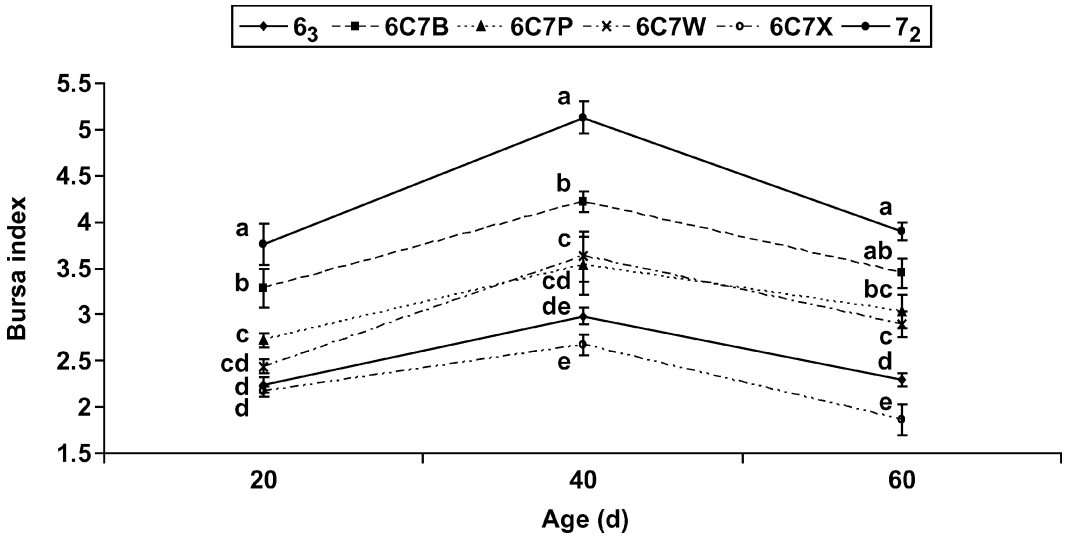

Figure 5. Relative bursa size of lines 6_3, 7_2, and 4 selected recombinant congenic strains at 20, 40, and 60 d of age observed in 2004 chickens. [a–e]The mean relative lymphoid organ size indices with no common letter are significantly different ($P < 0.05$). There were 9 to 11 chickens per age group per recombinant congenic strain.

Table 2. Sample sizes and lymphoid organ indices of the inbred parental lines and 4 of the recombinant congenic strains (RCS) replicated in 2004[1]

	No.		Bursa index		Thymus index		Spleen index		Bursa/thymus index	
Line	2002	2004	2002	2004	2002	2004	2002	2004	2002	2004
6_3	9	10	2.5 ± 0.05^{c}	2.3 ± 0.22^{d}	2.8 ± 0.10^{c}	2.7 ± 0.45^{d}	1.6 ± 0.06^{c}	1.6 ± 0.34^{c}	0.9 ± 0.05^{c}	0.9 ± 0.18^{c}
7_2	10	10	4.3 ± 0.10^{a}	3.9 ± 0.31^{a}	4.2 ± 0.07^{a}	4.6 ± 0.50^{a}	2.1 ± 0.05^{a}	2.3 ± 0.21^{a}	1.0 ± 0.03^{c}	0.8 ± 0.11^{c}
6C.7-B	6	9	4.5 ± 0.14^{a}	3.5 ± 0.48^{b}	3.8 ± 0.19^{b}	5.2 ± 0.35^{b}	1.5 ± 0.02^{c}	1.7 ± 0.17^{b}	1.2 ± 0.10^{bc}	0.7 ± 0.06^{c}
6C.7-P	20	10	3.5 ± 0.07^{b}	3.0 ± 0.56^{c}	2.3 ± 0.13^{c}	2.8 ± 0.35^{d}	1.6 ± 0.03^{c}	1.4 ± 0.25^{c}	1.6 ± 0.10^{a}	1.1 ± 0.25^{a}
6C.7-W	13	9	3.3 ± 0.11^{b}	2.9 ± 0.42^{c}	2.3 ± 0.17^{c}	3.2 ± 0.28^{d}	1.8 ± 0.04^{b}	1.6 ± 0.26^{c}	1.5 ± 0.12^{ab}	0.9 ± 0.13^{b}
6C.7-X	6	11	2.6 ± 0.12^{c}	1.9 ± 0.57^{e}	2.8 ± 0.15^{c}	3.1 ± 0.75^{c}	1.8 ± 0.04^{b}	1.6 ± 0.21^{c}	0.9 ± 0.04^{c}	0.6 ± 0.15^{d}

[a–d]Means within a column with no common superscript letter are statistically different based on Duncan's multiple range test at $P < 0.05$.

[1]All index estimates are presented as mean ± SE. The 2002 data are from chickens of 60 to 69 d of age, and the 2004 data are from chickens of 60 d of age.

5; Table 2). From 20 to 60 d, line 7_2 had a larger BF than line 6_3. The RCS 6C.7B also had a larger relative BF than line 6_3, as did 6C.7P at 20 and 60 d of age. The BF in RCS 6C.7W was comparable to line 6_3 at 20 d but was larger than line 6_3 at 40 and 60 d. The BF in 6C.7X was comparable to line 6_3 at 20 and 40 d but smaller than line 6_3 at 60 d. The 6C.7B consistently had the largest relative BF among the selected RCS and was clearly repeatedly different from 6C.7X at all the ages.

Regarding the T, at 20 d of age line 7_2 had a significantly larger relative T weight than RCS 6C.7B (Figure 6). The RCS 6C.7B had a larger T than line 6_3 and the other 3 RCS (6C.7X, -W, -P). At 40 and 60 d of age, comparable results were seen, although at 60 d 6C.7B had a larger thymus than line 7_2.

The BF/T index was comparable between lines 6_3 and 7_2 at 20, 40, and 60 d of age (Figure 7). However, the RCS 6C.7P, -W, and -B had a higher index than 6C.7X at 20 d of age. At 40 and 60 d of age 6C.7P and 6C.7W repeatedly had a higher BF/T index than 6C.7B and 6C.7X.

The spleen index was significantly larger in line 7_2 than line 6_3 and the 4 RCS at 20, 40, and 60 d of age (Figure 8). At 20 and 60 d, line 6_3 and the 4 RCS were comparable, and but at 40 d 6C.7B had a larger spleen than line 6_3 and 6C.7P ($P < 0.01$).

DISCUSSION

The relative size of the BF and T was larger in line 7_2 chickens than in line 6_3 chickens as described previously (Lee et al., 1981). As expected, most of the 6C.7 RCS resembled recurrent breeder line 6_3 in BF and T size at 60 to 69 d of age. However, lines 6C.7A and 6C.7B had a larger relative size of the BF and T that was similar to line 7_2. Analyses of 4 RCS at 20, 40, and 60 d of age confirmed that RCS 6C.7B had a larger relative BF and T than RCS 6C.7X, -W, or -P. It was concluded that RCS 6C.7A and 6C.7B are genetically similar to line 7_2 for weight of the BF and T. These data suggested that some gene(s) influence in unison the size of both the BF and T.

The relative weight of the BF tended to be about equal to the T within lines 6_3 and 7_2, as well as in most 6C.7 RCS, i.e., the BF/T index did not differ significantly from 1. An exception was seen in the 60- to 69-d-old 6C.7P and 6C.7W RCS males as they had a larger relative weight of the BF than T resulting in a BF/T index that was higher

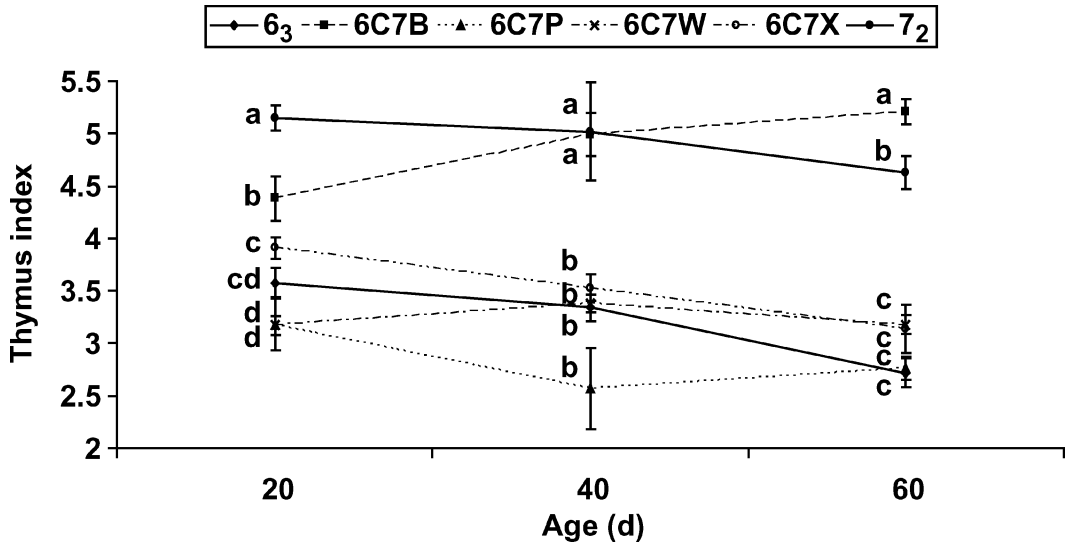

Figure 6. Relative thymus size index of the 2004 chickens. There were 9 to 11 chickens per age group per recombinant congenic strain. [a–d]The mean relative lymphoid organ size indices with no common letter are significantly different ($P < 0.05$).

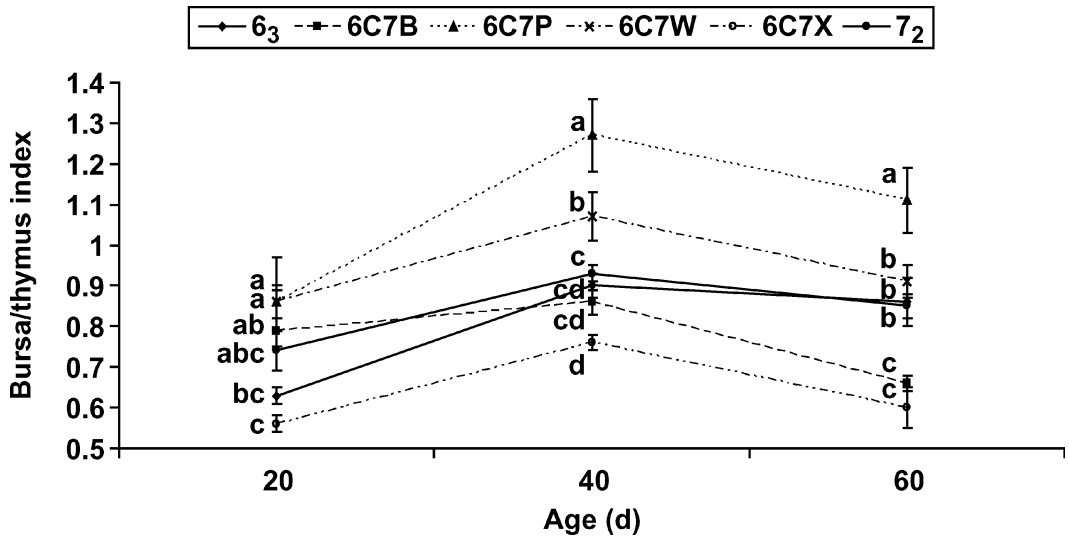

Figure 7. Bursa/thymus index of the 2004 chickens. There were 9 to 11 chickens per age group per recombinant congenic strain. [a–d]The mean relative lymphoid organ size indices with no common letter are significantly different ($P < 0.05$).

than seen in other strains. When the analysis of the BF and T size in 4 RCS (6C.7 B, -P, -W, -X) and lines 6_3 and 7_2 was repeated at 20, 40, and 60 d of age, RCS 6C.7P and 6C.7W again had the highest BF/T ratios at all ages. At 40 and 60 d of age RCS 6C.7P had a significantly higher BF/T ratio than any other line. These data suggested that although some genes influence the weight of both the BF and T as suggested above, other gene(s) can influence the weight of the BF independent of the T. Prior evidence exists for genes that influence the weight of the BF independent of the T. A line selected for high immune response to SRBC had a larger BF, but smaller T, than observed in a line selected for low immune response to SRBC (Ubosi et al., 1985).

It is easy to extract and assess the weight and maturation of the T in mammals since it is a 1-piece organ rather than organs consisting of fourteen lobes as in the chicken. On the other hand, a compact BF-type organ does not exist in mammals. Therefore, most attempts to determine genes regulating the size of the primary lymphoid organ are for the T size in mice (Ivanyi et al., 1972; Gregorova and Ivanyi, 1976; Peleg and Nesbitt, 1984; Hsu et al., 2003) and rats (Murakumo et al., 1996; Saito et al., 1997; Sharma et al., 1997). The QTL mapped to chromosomes 3 and X influenced the initial T cell count, and other loci on chromosomes 9 and 10 influenced the rate of thymic involution in recombinant inbred mouse strains. Importantly, some of these QTL have also been associated with im-

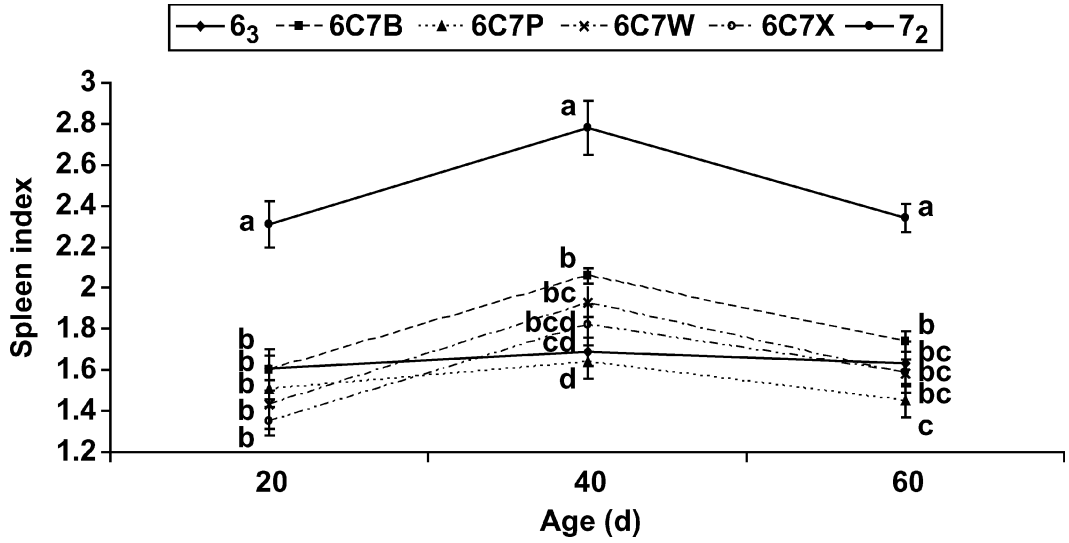

Figure 8. Relative spleen size of the 2004 chickens. The letter(s) on the left side of each data point indicate statistical significance. [a–d]The mean relative lymphoid organ size indices with no common letter are significantly different ($P < 0.05$). There were 9 to 11 chickens per age group per recombinant congenic strain.

mune regulation and autoimmune disease (Hsu et al., 2003). In the rat, linkage studies have identified loci responsible for enlargement and suppression of T size on chromosomes 1, 3, and 13 (Murakumo et al., 1996; Saito et al., 1997; Sharma et al., 1997). These studies in mice and rats suggested T size relative to body weight might be genetically modulated by alleles at a relatively small number of loci. The first chicken genome assembly was released in February 2004 (Hillier et al., 2004), and the second assembly is expected to be released early in 2006. Furthermore, a rich SNP database is also available in the chicken for QTL mapping or SNP association studies (Ka-Shu Wong et al., 2004). The present authors, along with others, have selectively validated 2,733 SNP approximately evenly spread throughout the chicken genome for samples from lines including the inbred lines 6_3, 7_2, and nineteen 6C.7 RCS. Hundreds of informative SNP have been identified on majority of the chromosomes covering 96% of the genome (data not shown). Thus the requirements for identifying genes governing the weight of BF or T in chicken exist, and an investigation can now be initiated.

The relative weight of the BF and T in the 2004 analysis of lines 6_3 and 7_2 and 4 RCS showed that the size of these organs increased from 20 to 40 d of age and was declining by 60 d of age. This is in general agreement with prior studies (Glick et al., 1956; Glick and Dreesen, 1967; Naukkarinen and Sorvari, 1984; Kreukniet, 1995). The 2002 study used 6_3, 7_2, and RCS chicks 60 to 69 d of age where some involution of the BF and T may have began. However, results at 60 d of age reflected those at 40 d of age in 2004. Therefore, the evaluation of excess males was acceptable as it allowed us to evaluate the lines when it was not possible to obtain large numbers of chicks. Six additional 6C.7 RCS had too few chicks for evaluation at 60 to 69 d of age in 2002, and these remain for evaluation. The BF and T cells were not isolated and evaluated because of the large number of chickens involved in these experiments. However, in other studies the relative size of the primary lymphoid organ is highly correlated with the number of lymphoid cells present in the organ. In the mouse T, there is a high correlation between relative T weight and thymocyte count (Hsu et al., 2003). Thus, it is likely that the genes regulating the relative weight of the T or BF are defining the number of lymphoid cells in that organ.

Lines 7_2 and RCS 6C.7A and 6C.7B had the largest BF and T in the current study. For several years mature hens from RCS 6C.7A and 6C.7B also possessed a greater quantity of IgG in their serum than hens of the other 6C.7 RCS (Yonash et al., 2002). This is consistent with the strong correlations found between the average relative weight of the BF as defined in the current study, and the average levels of IgG expression defined previously in these lines (Yonash et al., 2002). A moderately high correlation also existed between size of T and IgG levels. The principle function of the BF is the production of B lymphocytes, which in turn produce IgG, so it is plausible a greater mass of BF tissue may yield an increase in the production of IgG. Previous studies of Single Comb White Leghorn families have shown a correlation between BF size and antibody production (Glick et al., 1956). Families selected for increased BF weight produced higher antibody levels responding to *Vibrio fetus* bacteria than families selected for small bursa (Sadler and Glick, 1962). However, this is not a consistent relationship. For example, selection for increased BF weight in broilers (Temple and Jaap, 1961) did not affect antibody titer after a challenge with *Salmonella typhimurium* (Jaffe and Jaap, 1966).

In an initial study of the 6C.7 RCS for MD the 6C.7 M, -P and -W RCS were found to be moderately more susceptible to tumors after infection with the JM strain of MD virus (Bacon et al., 1996; Yonash et al., 1998). In the present study RCS 6C.7P and W had a high BF/T ratio. This result in conjunction with earlier experiments (Lee et al., 1981) suggested there might be a correlation between primary lymphoid organ size and susceptibility to MD tumors. Clearly more experiments using larger numbers of chicks and possibly a more differentiating MD virus are needed. The number of laying hens in each 6C.7 RCS has recently been increased to 18 to 21 in hopes of producing adequate numbers of chicks needed for studies on chicks of these 6C.7 RCS for a given trait.

The spleen is the principle secondary lymphoid organ in the chicken and is important in all immune responses. The spleen is greatly influenced by environmental challenges. Therefore, spleen size is determined by the environmental as well as genetic factors. Relative spleen weight is easy to obtain, so this was also evaluated in 2002 and 2004. In 2002, line 7_2 had a larger spleen than line 6_3 at 60 to 69 d of age, and this was repeatedly seen in 2004 at 20, 40, and 60 d of age. These data confirmed earlier results (Lee et al., 1981). At 60 to 69 d of age, 6C.7C was the only RCS with a spleen weight equivalent to line 7_2, and the other 6C.7 RCS were comparable with line 6_3. However, RCS 6C.7C has not been analyzed a second time. These results confirmed that lines 6_3 and 7_2 differ in relative spleen weights when chickens are grown in the same environment and indicated that RCS 6C.7C may be useful for evaluating genes influencing spleen size.

The results from the current study of lines 6_3 and 7_2 and 13 RCS lead to the conclusion that the size of the BF and T are generally comparable within a chicken strain. In 2 RCS the size of the T and BF were comparatively large as in line 7_2, leading to the conclusion that allelic forms at 1 or more loci in these RCS regulate the size of both organs. In 2 other RCS the BF was large compared with the T suggesting that allelic forms at some loci in these RCS influence the BF independent of the T. There were correlations between the average size of the BF and T of a RCS as defined in the present study, and other traits previously analyzed in these RCS, e.g., concentration of IgG in plasma of adult hens, and resistance to MD tumors. The evaluation of genome variability of these lines is underway, and the RCS are available for research on traits that differ between lines 6_3 and 7_2.

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